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Anti-inflammatory, anti-apoptotic, and antioxidant effects of obestatin on the colonic mucosa following acetic acid–induced colitis

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Abstract

Background: Cellular inflammatory processes, fibrogenesis, and apoptosis are the most characteristic pathologic features of colonic injury and colitis in human and experimental animals. Obestatin, a peptide derived from proghrelin, is reported to have significant protective and curative actions on many gastrointestinal tract inflammatory diseases, including ulcerative colitis (U.C.). However, its exact protective mechanisms and the associated histopathological changes, are still in need of deeper exploration. This study explores the effect of obestatin on the course of acetic acid (A.A.)-induced colitis as an antifibrotic, anti-inflammatory, and anti-apoptotic agent in relation to associated tissue stress parameters.

Materials and methods: A total of 40 healthy male albino Wistar rats weighing 200–250 g were recruited in this study. The rats were classified into four groups (10 rats each); group I control, group II obestatin only treated (16 nmol/kg), group III, colitis induced group (acetic acid 1 mL of 3.5% (v/v), and group IV, (AA-induced colitis + obestatin) for 14 days. Colonic samples were examined after staining hematoxylin and eosin, Alcian blue, Masson trichrome. The expression of PCNA, NFkB, and Caspase-3 was estimated after immunohistochemical staining. Oxidative stress parameters, antioxidant enzymes, tissue myeloperoxidase (MPO) activity, ghrelin, and fibrogenesis markers were identified by immunoassay and colorimetric techniques.

Results: Colonic mucosa of group IV exhibited mucosal healing and regeneration of the surface epithelium with the restoration of the goblet cells' function together with a decline in PCNA, NFkB, and Caspase-3 immunoreactivity in comparison to group III. This was accompanied by a reduction of the expression of fibrosis markers; Hydroxyproline and Fibronectin. In addition, tissue antioxidant status was significantly improved with a marked reduction of tissue MPO. Ghrelin level was significantly increased in comparison to group III. Group IV exhibited significant reduction in the levels of oxidative stress markers; MDA, TOS with a marked increase in the activity of antioxidant enzymes, SOD, CAT, and total cellular TAC.
Conclusions: The concomitant treatment of obestatin inhibits the development of AA-induced colitis. The data signify that it has both curative and protective effects via antifibrotic, antioxidant, and anti-inflammatory activities.
Key words: ghrelin, ulcerative colitis, oxidative stress, colon histopathology, apoptosis

INTRODUCTION

Ulcerative colitis (U.C.) is a common inflammatory bowel disease (IBD) mediated by an autoimmune process that significantly leads to extensive mucosal disruption and ulceration [42]. Despite innumerable research in the field of IBD, the exact etiology of U.C. is still unclear, and their pathogenesis is complex, requiring the co-existence of environmental and genetic factors. However, research has suggested that both U.C. and Crohn's disease result from an abnormal immunological response to microflora present in the digestive system [39]. Untreated U.C. may proceed to colorectal cancer, life-threatening respiratory and/or circulatory complications [16]. The severity of the disease is associated with the extent of colonic barrier disruption with subsequent exposure of the mucosa and submucosa to proinflammatory and environmental factors [45, 46, 49, 58]. These factors can directly or indirectly activate local mesenchymal cells, such as fibroblasts, myofibroblasts, and smooth muscle cells, for the expression of increasing amounts of extracellular matrix components (ECM) such as collagens or fibronectins. These proceedings eventually end in fibrosis, goblet cell inhibition, and disruption of the colonic wall histological building [38, 45, 52]. In addition, the associated imbalance between tissue matrix-metalloproteinases, which degrade ECM, and tissue inhibitors of metalloproteinases, which inhibit ECM degradation, adds to the alteration of the colonic wall construction [38].

The serious complications of U.C. and the lack of presence of proper drug therapy necessitates more scientific trials to choose and/or discover alternative drug candidates with improved tolerability, minimum side effects, and more safety for the treatment of this disease. There are numerous methods to ameliorate the signs and symptoms of IBD, but there is no method to permanently cure this disease [2]. This leads to a search for new therapeutic strategies.

Obestatin is reported in many studies to possess protective and therapeutic effects on gastrointestinal tract inflammatory diseases [9, 10, 13]. Chemically, obestatin is a ghrelin-associated peptide formed of a 23-amino acid peptide derived from the post-translational processing of the preproghrelin gene [61]. Biologically, obestatin was identified in several organs such as the stomach, endocrine pancreas, adipose tissue, the lung, liver, skeletal muscle, mammary glands, and the male reproductive system [23, 26, 40]. Nevertheless, the stomach comprises the major source of circulating obestatin [14, 23].-

In experimental models, administration of obestatin exhibited numerous therapeutic effects mediated through activation of receptors like the G-protein-coupled receptor [60] and the glucagon-like peptide 1 receptor in adipocytes and pancreatic beta cells [24, 25]. The main reported effects were suppression of the motility of the gastrointestinal tract, inhibition of cellular oxidative stress induced by molecular H_2O_2 [57], and regulation of the secretion of insulin [24, 25]. Accordingly, obestatin was considered a promising therapeutic agent in managing many clinical syndromes [27, 35, 62], particularly those related to the digestive system [47, 57]. Moreover, the role of obestatin in augmenting the healing of gastric ulcers via increasing both blood flow in gastric mucosa and cell proliferation has been reported [17]. Inflammatory bowel disease (IBD) is another example with the advantage of using the ratio of obestatin to ghrelin in the serum as a corresponding marker for monitoring the severity of the accompanying inflammatory process [2, 30].

Experimentally development of colitis and related gastric complications induced by acetic acid [39] or dextran sodium sulfate [43] were significantly suppressed following administration of obestatin. Even though these studies proved the therapeutic effect of obestatin, the exact protective mechanisms, and the associated histopathological changes, are still in need of deeper exploration.

From the aforementioned studies relating to the pathogenesis of U.C. and the proposed therapeutic effect of obestatin, this study aims to explore the biological roles of obestatin as an

antifibrotic, anti-inflammatory, and antioxidant agent on a murine model of acetic acid (A.A.)-induced colitis, and to investigate whether this action is associated with changes in the expressions of PCNA, NFkB, and Caspase-3, and further to explore the concomitant changes in the levels of oxidative stress parameters, antioxidant enzymes, tissue myeloperoxidase (MPO) activities. The expressions of ghrelin and fibrogenic markers like collagen and fibronectins in mucosal tissues of control and in (A.A.)-induced colitis rats are also pursued.

MATERIAL AND METHODS

A total of 40 healthy male albino Wistar rats weighing 200–250 g were obtained from the Animal breeding unit at (Taibah University). All the study steps were strictly designed and followed by the Ethical Committee of Taibah University Research Board and were in line with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication 85-23 Rev. 1985).

All animals were kept in cages at room temperature under a 12 h light-dark cycle. Prior to induction of colitis, the animals were fasted for 16 h with free access to water through ad libitum. Then, the animals were provided with normal basal diets containing 21.1% of protein, 5.1% of fat, 60.0% of carbohydrates, 3.9% of fiber, 7.9% of minerals, and 2.0% of vitamins.

The rats were randomly categorized into four groups (10 rats each). Group I (control) was treated with saline, group II (treated with obestatin only), group III (AA-induced colitis), and group IV (AA-induced colitis + obestatin). Starting 24 h before the rectal enema, the rats were treated with intraperitoneally (i.p.) saline (groups I, II & III) or obestatin (group IV) twice per day for 4 consecutive days. Rat obestatin (Yanaihara Institute, Shizuoka, Japan) was given at dose of 16 nmol/kg [13, 17, 39].

After pentobarbital (30 mg/kg i.p.) anesthesia, 1 mL of 3.5% (v/v) acetic acid diluted in saline was used to induce colitis in groups III and IV by a rectal enema using a polyethylene catheter containing acetic acid solution inserted into the rectum at 4.5 cm from the anus. Rats were kept in a vertical position during installation and for 1 min after installation to avoid solution leakage. Animals of groups I and II received 0.5 ml of saline 0.9% by the same procedure.

Fourteen days after the induction of colitis, rats were again anesthetized with pentobarbital (30 mg/kg i.p.) and sacrificed to collect the serum and colon tissue samples from all groups for

biochemical and histopathological investigations. After excision of the colon, it was examined thoroughly for microscopic, immunohistochemical, and biochemical examinations.

The colon samples were fixed in 10% buffered formaldehyde and embedded in paraffin. Paraffin sections were processed and stained with hematoxylin and eosin, Alcain blue, and Masson trichrome stains according to the standard methodology. The stained sections were examined under a light microscope (Nikon Eclipse E600W, Japan), and the represented areas were photographed.

The hematoxylin and eosin-stained sections were further examined by the pathologist, uninformed about the treatment given. The grading of colonic damage (ulceration, inflammation, depth of the lesion, and fibrosis) and the number of goblet cells were determined using a scale of Vilaseca as described in detail previously [37, 54]. The standard techniques of staining of Caspase-3, PCNA, and NFkB were followed as provided by the instructors [1, 18, 59].

The homogenates of colonic tissue were subjected to the estimation of oxidative stress antioxidant system markers, including malondialdehyde (MDA), total oxidant status (TOS), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) levels as previously analyzed [19, 50].Tissue myeloperoxidase (MPO) activity was identified in colonic tissue samples by colorimetric assays as previously reported in the literature [61]. The amount of MPO present in tissue samples that caused a change in absorbance measured at 460 nm for 3 minutes referred to one unit of MPO enzyme activity [28]. Ghrelin concentrations were estimated in the homogenates of the colonic tissue samples using commercially available EIA assay kits (Phoenix Pharmaceuticals, Burlingame, CA) as fully characterized in previous work by Ghomraoui et al. [22]. Hydroxyproline was estimated in acidified colonic tissues by adding 5mL of 6N HCl for 3 hours at 130°C, as reported previously in 1963 by Bergman et al. [6]. For assessment of fibronectin, a glass homogenizer was used to homogenize colonic tissues with 5-10 mL of ice-cold PBS (0.01mol/L, pH 7.0-7.2). An ultrasonic cell disrupter was applied to the resulting suspension to destroy hard cell wall compartments for future extraction. Then, centrifugation of the homogenates for 5 minutes at 5000×g and the residue was used using an immunoassay ELISA kit (ABIN1874233, Atlanta, GA30338, USA) at a wavelength of 450 nm on a spectrophotometer. The fibronectin concentration was then determined by comparing the O.D. of the samples to the standard curve as described by Attallah et al. [4].

Statistical analysis

All data were expressed as Mean ± Standard Deviations (S.D.). The results obtained were statistically analyzed by GraphPad Prism (version 7). In addition, a one-way ANOVA test followed by Tukey's post hoc analysis was applied to compare and identify the significance between groups. The statistical significance was assigned at a p-value <0.05.

Results

In groups, I and II, tissue sections of rats exhibited normal colonic structure with intact colonic mucosa and numerous goblet cells on the surface epithelium and crypts (Figs. I.A. & B). Notable pathological changes present in group III involved all colonic laminae (epithelial, propria, muscularis mucosae, and the submucosa). These laminae could not be distinguished from each other due to extensive inflammatory cell infiltration and ulceration ((Figs. I.C.). In addition to severe goblet cell depletion, an absence of crypts was evident. Treatment with obestatin in group IV did not completely alleviate the lesions but just restricted them. (Fig. 1D). The mucosa retained its unharmed appearance with active goblet cells detected on the surface and the crypt’s epithelium.

The mucous secretions of the goblet cells in groups I & II stained Alcian blue stain demonstrated numerous deeply stained goblet cells lining the crypts and an intact film of mucus on the surface epithelium (Fig. 2A & B). On the other hand, Group III shows nearly absent goblet cells and mucous, as evidenced by an extreme reduction in the intensity of Alcian blue stain. Group IV revealed few goblet cells and restored the intensity of the stain, particularly on the surface (Fig. 2C & D).

Masson trichrome stain of groups I & II detect minimal bluish stained collagen fibers in the lamina propria, muscularis mucosae, and the submucosa (Fig.3 A & B). Group III, on the other hand, showed increased expression of fibrosis in all colonic laminae (Fig.3C). Group IV still shows extensive collagen fibers expression (Fig.3 D).

The immunohistochemical examination of PCNA of the mucosa of the colon of group I exhibited weak brown cytoplasmic positive immunoreaction in the epithelium and few cells in the lamina propria (Fig.4 A). the picture was more or less the same in group II (Fig. 4 B). Group III exhibited marked increased immunoreactivity in both epithelial cells and cells of lamina propria (Fig. 4 C). On the other hand, sections in the colonic mucosa of group IV demonstrated a decrease in positive immunoreaction compared to groups III (Figure 4 D).

NFkB immune staining of the group I demonstrated scanty brownish cytoplasmic positive immunoreaction in both the surface epithelium and the cells of lamina propria (Fig.5 A). Group II showed a similar presentation (Fig. 5B). Whereas sections of group III disclosed widespread marked immunoreactivity in all layers of colonic mucosa (Fig. 5C), while colonic mucosa of group IV displayed a limited presentation of the immunoreactivity to the mucosa and nearly absent reaction in the lamina propria (Figure 5D).

Caspase 3 expression in the colon mucosal area showed a nearly absent reaction in both groups I & II (Fig. 6 A & B). Group III showed a strong and diffuse reaction in all layers of the colonic mucosa (Fig. 6 C). Group IV shows very weak reactivity in comparison (Fig.6 D).

The grading of colonic injury and a number of goblet cells was scored in table I. The results showed that normal rats treated with saline or obestatin showed no mucosal damage. However, rats with colitis induced by A.A. (group III) showed significant mucosal damage and remarkably decreased goblet cells number compared to control or obestatin-treated rats (group I & II). Group IV, on the other hand, showed a significant improvement in the colon mucosal damage score compared to group III rats. Additionally, the number of goblet cells was significantly increased in group IV compared with group III.

The expression levels of MDA, TOS, and the activity of SOD, CAT, and TAC in tissue samples of group I and rats of the experimental groups are tabulated (table II). In groups I and II, the results showed no significant effect. However, group III showed a significant reduction in the concentration of MDA, TOS, and an increase in the activity of SOD, CAT, and TAC, respectively. In group III, A.A. induced a significant increase in the levels of MDA, TOS and a reduction in the activity of SOD, CAT, and TAC compared to groups I & II. In rats with colitis treated with obestatin (group IV), there was a significant ($P< 0.003$) reduction in the levels of MDA, TOS with more increased in the activity of antioxidant enzymes, SOD, CAT, and total cellular TAC.

The expression levels of tissue ghrelin and MPO is shown in graph 1. A significant increase in the tissue activity of MPO and a decrease in ghrelin levels in group III rats compared to control rats treated with saline only. In AA-induced colitis rats treated with obestatin (group IV), a significant reduction in the activity of MPO with an elevation in the levels of tissue ghrelin. A significant increase in the expression levels of fibronectin and hydroxyproline as markers of fibrosis. (graph 2) was noted in group III compared to groups I and II. However, when treated

with obestatin compared to group III, rats of group IV exhibited a significantly reduced expression of both fibronectin and hydroxyproline.

DISCUSSION

The current study results established the curative and protective effect of i.p. obestatin on the histopathological structure of the colon and the mucosal inflammatory and cellular antioxidant levels.

The model of colitis induced by acetic acid (A.A.) investigates common colon diseases such as IBD as it induces a similar picture of inflammation together with ulceration [55]. The models of AA-induced colitis inject the acid with the tip of the catheter positioned at variable distances (from 1.2 [36] to 8 cm [44]) proximal to the anus verge. In this study, an intermediate depth of catheter insertion at 4.5 cm from the anus verge was chosen.

The curative outcome of obestatin was confirmed on the histopathological level as obestatin significantly amended the lesions grades, ulcerations, inflammatory cell infiltrations, and the loss of crypts induced by A.A. The number of the goblet cells was significantly increased, and goblet cells were redetected with increased mucous secretions on the surface and crypt epithelium as evidenced by Alcian blue stain when compared with the AA-induce UC as likewise reported [31]. Obestatin significantly reduced colitis by increasing blood leukocytes and mucosal concentration of proinflammatory markers and leading to reduced histological signs of colonic damage [53]. These results also showed that obestatin improved the mucosal blood flow in the colon and decreased the local and systemic inflammatory processes.

As a marker of cell proliferation, PCNA immune stain was markedly increased in AA-induced colitis and decreased with obestatin. Similar results were reported [53]. In NFkB immune stain as a marker of coordination of chronic inflammatory condition of the colon also reported a marked increase in the AA-induced colitis and an apparent decrease in NFkB immunoreactivity with the use of obestatin indicating tissue induced anti-inflammatory effect [5]. At the same time, the indicator of apoptosis, Caspase-3, was also markedly decreased in the obestatin treated group after induction of U.C. compared to the AA-induced colitis group. This indicator was helpful in a study on the heart proving the protection of the cardiac muscles with prior use of obestatin [3].

The administration of obestatin improved antioxidant status, reduced inflammation, increased the expression levels of tissue ghrelin, and reduced the activity of tissue MPO. At the same time, this curative effect was proved in AA-induced colitis, whereas fibrosis markers, inflammatory MPO markers, and oxidant markers such as MDA, TOS were significantly reduced, and the antioxidant enzymes SOD, CAT, and total TAC were significantly increased. The objective antifibrotic impact of obestatin was proven in our study by the marked inhibited expression of Masson trichrome stain in the treated group compared to the AA-UC group. These results are in line with others who reported that obestatin displayed preventive and therapeutic effects on acute pancreatitis [9, 10, 12], chronic gastric ulcers [17], and some models of colitis [39].

Oxidative stress is an important component of mucosal injury pathogenesis [32] which activates cellular apoptosis [21, 51], as evidenced in our study by the increased expression of Caspase-3 as a marker of apoptosis. In contrast, deregulation or excessive apoptosis disrupts equilibrium and manifests variable clinical presentations [41, 48]. That is why most studies have focused on proposed treatment modalities with antioxidant and anti-inflammatory properties.

Colonic injury in this study was detected in the AA-colitis model with a significant increase in oxidative stress markers and histopathological changes. Oxidative stress is the key factor of AA-associated UC, where the overload of oxygen-free radicals induces disruption and apoptosis [15]. In addition, A.A. significantly augmented the reduction in the level of the antioxidant enzyme. Similarly, a decrease in the activity of tissue SOD, CAT, and the total cellular TAC was reported in rats with AA-colitis [20]. The inhibition of lipid peroxidation and improvement of cellular antioxidant status is one of the anti-inflammatory actions of obestatin and ghrelin as previously described [29, 34].

Ghrelin, an alternative product of preproghrelin, also had a therapeutic effect on the gastrointestinal tract [56]. It has a protective effect on gastrointestinal mucosa against damage caused by harmful factors [8, 29, 33] and potentially inhibits colitis development. Ghrelin has a protective effect on gastrointestinal mucosa against damage caused by harmful factors [7, 56] and potentially inhibits the development of colitis. Tissue ghrelin levels were identified to confirm the protective effect of obestatin against AA-UC and confirmed a significantly increased tissue ghrelin level in concomitant to the previously reported protective effect of ghrelin on gastrointestinal mucosa against damage with harmful factors [7, 29, 33].

In our study, the expression of tissue MPO was reported to be directly proportional to the number of inflammatory cell infiltration [11, 43]. It was suggested that obestatin inhibits polymorphonuclear cell recruitment during the progression of chronic colitis. Obestatin decreased tissue MPO levels significantly in rats with AA-colitis in the current study. At the same time, the histopathological examination reported that the inflammatory cell infiltration was markedly reduced. Accordingly, our results with others provided further evidence that obestatin exhibits anti-inflammatory actions and confirmed its curative, protection, and healing properties in this model.

Limitations of the study

We used Wistar rats as they are good models when studying colitis. However, some of the causes behind controversy may be the animal or species-related difference and this is one of the study limitations. Moreover, the effect related to multiple doses and long term use of acetic acid and obestatin may cause differences in results. Furthermore. The sex difference in the inflammatory response may be cause controversial results. We used male animals because although females have higher innate and adaptive immune responses than males, this contributes to increased susceptibility to inflammatory and autoimmune diseases in females compared with males. So it is recommended to repeat the experiment with multiple doses of drugs and with different animals and species with different sex.

Clinical applications

We recommend the use of obestatin in management of colitis through its anti-inflammatory, antioxidant, antiapoptotic and antifibrotic effect with regulation of regeneration of colic mucosa.

CONCLUSIONS

The current study concludes that pretreatment with obestatin at a dose of 16 nmol/kg inhibits the development of acetic acid-induced colitis. This effect significantly proceeded via a reduction in cellular inflammatory and overproduction of both cellular fibrosis and oxidative stress markers with a potential improvement of antioxidant status of the colonic cells. These data recommended that obestatin was independent anti-inflammatory, antioxidant, and antifibrotic against A.A.- induced colitis.

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REFERENCES

1. Al-Henhena N, Ying RPY, Ismail S, Najm W, Khalifa SA, El-Seedi H, et al. Chemopreventive efficacy of Andrographis paniculata on azoxymethane-induced aberrant colon crypt foci in vivo. 2014;9(11):e111118.
2. Alexandridis E, Zisimopoulos A, Liratzopoulos N, Katsos I, Manolas K, Kouklakis GJIbd. Obestatin/ghrelin ratio: a new activity index in inflammatory bowel diseases. 2009;15(10):1557-61.
3. Alloatti G, Arnoletti E, Bassino E, Penna C, Perrelli MG, Ghé C, et al. Obestatin affords cardioprotection to the ischemic-reperfused isolated rat heart and inhibits apoptosis in cultures of similarly stressed cardiomyocytes. 2010;299(2):H470-H81.
4. Attallah AM, Abdallah SO, Attallah AA, Omran MM, Farid K, Nasif WA, et al. Diagnostic value of fibronectin discriminant score for predicting liver fibrosis stages in chronic hepatitis C virus patients. 2013;12(1):44-53.
5. Beck EA, Currey MC, Small CM, Cresko WAJGG, Genomes, Genetics. QTL mapping of intestinal neutrophil variation in threespine stickleback reveals possible gene targets connecting intestinal inflammation and systemic health. 2020;10(2):613-22.
6. Bergman I, Loxley RJAc. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. 1963;35(12):1961-5.
7. BRZOZOWSKI T, KONTUREK P, SLIWOWSKI Z, DROZDOWICZ D, KWIECIEN S, PAWLIK M, et al. NEURAL ASPECTS OF GHRELIN-INDUCED GASTROPROTECTION.
8. Brzozowski T, Konturek P, Sliwowski Z, Drozdowicz D, Kwiecien S, Pawlik M, et al. Neural aspects of ghrelin-induced gastroprotection against mucosal injury induced by noxious agents. 2006;57:63-76.
9. Bukowczan J, Warzecha Z, Ceranowicz P, Kuśnierz-Cabala B, Tomaszewska R, Dembinski AJEjop. Pretreatment with obestatin reduces the severity of ischemia/reperfusion-induced acute pancreatitis in rats. 2015;760:113-21.
10. Bukowczan J, Warzecha Z, Ceranowicz P, Kuśnierz-Cabala B, Tomaszewska RJPO. Obestatin accelerates the recovery in the course of ischemia/reperfusion-induced acute pancreatitis in rats. 2015;10(7):e0134380.
11. Ceranowicz P, Warzecha Z, Cieszkowski J, Ceranowicz D, Kuśnierz-Cabala B, Bonior J, et al. Essential role of growth hormone and IGF-1 in therapeutic effect of ghrelin in the course of acetic acid-induced colitis. 2017;18(6):1118.

12. Ceranowicz P, Warzecha Z, Dembinski A, Cieszkowski J, Dembinski M, Sendur R, et al. Pretreatment with obestatin inhibits the development of cerulein-induced pancreatitis. J Physiol Pharmacol. 2009;60(3):95-101. Epub 2009/10/15. PubMed PMID: 19826187.
13. Ceranowicz P, Warzecha Z, Dembinski A, Cieszkowski J, Dembinski M, Sendur R, et al. Pretreatment with obestatin inhibits the development of cerulein-induced pancreatitis. 2009;12(3):95.
14. Ceranowicz P, Warzecha Z, Dembinski AJJPP. Peptidyl hormones of endocrine cells origin in the gut—Their discovery and physiological relevance. 2015;66(1):11-27.
15. Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MAJTTjoem. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. 2005;206(2):131-9.
16. Chu TP, Moran GW, Card TRJJoCs, Colitis. The pattern of underlying cause of death in patients with inflammatory bowel disease in England: a record linkage study. 2017;11(5):578-85.
17. Dembiński A, Warzecha Z, Ceranowicz P, Cieszkowski J, Dembiński M, Ptak-Belowska A, et al. Administration of obestatin accelerates the healing of chronic gastric ulcers in rats. 2011;17(8):BR196.
18. Ercan G, Yigitturk G, Erbas OJAcb. Therapeutic effect of adenosine on experimentally induced acute ulcerative colitis model in rats1. 2020;34.
19. Erel OJCb. A novel automated method to measure total antioxidant response against potent free radical reactions. 2004;37(2):112-9.
20. Flora G, Gupta D, Tiwari AJIt. Toxicity of lead: a review with recent updates. 2012;5(2):47-58.
21. Franco R, Sánchez-Olea R, Reyes-Reyes EM, Panayiotidis MIJMRGT, Mutagenesis E. Environmental toxicity, oxidative stress and apoptosis: menage a trois. 2009;674(1-2):3-22.
22. Ghomraoui FA, Alotaibi ST, Alharthi MA, Asiri SS, Almadi MA, Alharbi OR, et al. Plasma ghrelin and leptin in patients with inflammatory bowel disease and its association with nutritional status. 2017;23(3):199.
23. Granata R, Baragli A, Settanni F, Scarlatti F, Ghigo EJJome. Unraveling the role of the ghrelin gene peptides in the endocrine pancreas. 2010;45(3):107-18.
24. Granata R, Gallo D, Luque RM, Baragli A, Scarlatti F, Grande C, et al. Obestatin regulates adipocyte function and protects against diet-induced insulin resistance and inflammation. 2012;26(8):3393-411.
25. Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, et al. Obestatin promotes survival of pancreatic β-cells and human islets and induces expression of genes involved in the regulation of β-cell mass and function. 2008;57(4):967-79.
26. Gurriarán-Rodríguez U, Santos-Zas I, Al-Massadi O, Mosteiro CS, Beiroa D, Nogueiras R, et al. The obestatin/GPR39 system is up-regulated by muscle injury and functions as an autocrine regenerative system. 2012;287(45):38379-89.
27. Harsch IA, Koebnick C, Tasi AM, Hahn EG, Konturek PCJDd, sciences. Ghrelin and obestatin levels in type 2 diabetic patients with and without delayed gastric emptying. 2009;54(10):2161-6.

28. Hillegass L, Griswold D, Brickson B, Albrightson-Winslow CJJopm. Assessment of myeloperoxidase activity in whole rat kidney. 1990;24(4):285-95.
29. Íşeri SO, Sener G, Yuksel M, Contuk G, Cetinel S, Gedik N, et al. Ghrelin against alendronate-induced gastric damage in rats. 2005;187(3):399-406.
30. Jung JY, Jeong JB, Kim JW, Kim SH, Koh S-J, Kim BG, et al. Circulating ghrelin levels and obestatin/ghrelin ratio as a marker of activity in ulcerative colitis. 2015;13(1):68.
31. Kaur K, Saxena A, Larsen B, Truman S, Biyani N, Fletcher E, et al. Mucus mediated protection against acute colitis in adiponectin deficient mice. 2015;12(1):1-9.
32. Kim YJ, Kim EH, Hahm KBJJog, hepatology. Oxidative stress in inflammation-based gastrointestinal tract diseases: Challenges and opportunities. 2012;27(6):1004-10.
33. Konturek PC, Brzozowski T, Walter B, Burnat G, Hess T, Hahn EG, et al. Ghrelin-induced gastroprotection against ischemia–reperfusion injury involves an activation of sensory afferent nerves and hyperemia mediated by nitric oxide. 2006;536(1-2):171-81.
34. Kwiecien S, Jasnos K, Magierowski M, Sliwowski Z, Pajdo R, Brzozowski B, et al. Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress-induced gastric injury. 2014;65(5):613-22.
35. Liu W, Yue H, Zhang J, Pu J, Yu QJCmj. Effects of plasma ghrelin, obestatin, and ghrelin/obestatin ratio on blood pressure circadian rhythms in patients with obstructive sleep apnea syndrome. 2014;127(5):850-5.
36. MacPherson B, Pfeiffer CJD. Experimental production of diffuse colitis in rats. 1978;17(2):135-50.
37. Maduzia D, Matuszyk A, Ceranowicz D, Warzecha Z, Ceranowicz P, Fyderek K, et al. The influence of pretreatment with ghrelin on the development of acetic-acid-induced colitis in rats. 2015;66(6):875-85.
38. Matsuno K, Adachi Y, Yamamoto H, Goto A, Arimura Y, Endo T, et al. The expression of matrix metalloproteinase matrilysin indicates the degree of inflammation in ulcerative colitis. 2003;38(4):348-54.
39. Matuszyk A, Ceranowicz P, Warzecha Z, Cieszkowski J, Bonior J, Jaworek J, et al. Obestatin accelerates the healing of acetic acid-induced colitis in rats. 2016;2016.
40. Moretti E, Vindigni C, Tripodi S, Mazzi L, Nuti R, Figura N, et al. Immunolocalisation of ghrelin and obestatin in human testis, seminal vesicles, prostate and spermatozoa. 2014;46(9):979-85.
41. Myers BS, Martin JS, Dempsey DT, Parkman HP, Thomas RM, Ryan JPJAJoP-G, et al. Acute experimental colitis decreases colonic circular smooth muscle contractility in rats. 1997;273(4):G928-G36.
42. Niu X, Zhang H, Li W, Wang Y, Mu Q, Wang X, et al. Protective effect of cavidine on acetic acid-induced murine colitis via regulating antioxidant, cytokine profile and NF-kappaB signal transduction pathways. Chem Biol Interact. 2015;239:34-45. Epub 2015/06/24. doi: 10.1016/j.cbi.2015.06.026. PubMed PMID: 26102009.

43. Pamukcu O, Kumral ZNO, Ercan F, Yegen BÇ, Ertem DJJopg, nutrition. Anti-inflammatory effect of obestatin and ghrelin in dextran sulfate sodium–induced colitis in rats. 2013;57(2):211-8.
44. Randhawa PK, Singh K, Singh N, Jaggi ASJTKjop, pharmacology. A review on chemical-induced inflammatory bowel disease models in rodents. 2014;18(4):279-88.
45. Rieder F, Fiocchi CJJoCs, Colitis. Intestinal fibrosis in inflammatory bowel disease—current knowledge and future perspectives. 2008;2(4):279-90.
46. Scales BS, Huffnagle GBJTJop. The microbiome in wound repair and tissue fibrosis. 2013;229(2):323-31.
47. Słupecka M, Woliński J, Herman AP, Ochniewicz P, Kornacka MKJMwr. Biological role of obestatin in physiology and pathophysiology. 2012;16(1):47-52.
48. Souza HS, Tortori CJ, Castelo-Branco MT, Carvalho ATP, Margallo VS, Delgado CF, et al. Apoptosis in the intestinal mucosa of patients with inflammatory bowel disease: evidence of altered expression of FasL and perforin cytotoxic pathways. 2005;20(3):277-86.
49. Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarström C, Loos T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. 2010;177(6):2804-15.
50. Sun Y, Oberley LW, Li YJCc. A simple method for clinical assay of superoxide dismutase. 1988;34(3):497-500.
51. Thompson CBJS. Apoptosis in the pathogenesis and treatment of disease. 1995;267(5203):1456-62.
52. Vaalamo M, Karjalainen-Lindsberg M-L, Puolakkainen P, Kere J, Saarialho-Kere UJTAjop. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. 1998;152(4):1005.
53. van den Toren SJ, van Grieken A, Mulder WC, Vanneste Y, Lugtenberg M, de Kroon ML, et al. School absenteeism, Health-Related Quality of Life [HRQOL] and happiness among young adults aged 16–26 years. 2019;16(18):3321.
54. Vilaseca J, Salas A, Guarner F, Rodriguez R, Martinez M, Malagelada JJG. Dietary fish oil reduces progression of chronic inflammatory lesions in a rat model of granulomatous colitis. 1990;31(5):539-44.
55. Vishwakarma N, Ganeshpurkar A, Pandey V, Dubey N, Bansal DJDD. Mesalazine–probiotics beads for acetic acid experimental colitis: formulation and characterization of a promising new therapeutic strategy for ulcerative colitis. 2015;22(1):94-9.
56. Warzecha Z, Dembinski AJCmc. Protective and therapeutic effects of ghrelin in the gut. 2012;19(1):118-25.
57. Xing Y-X, Yang L, Kuang H-Y, Gao X-Y, Liu H-LJN. Function of obestatin in the digestive system. 2017;34:21-8.
58. Yamagata M, Mikami T, Tsuruta T, Yokoyama K, Sada M, Kobayashi K, et al. Submucosal fibrosis and basic-fibroblast growth factor-positive neutrophils correlate with colonic stenosis in cases of ulcerative colitis. 2011;84(1):12-21.

59. Zedan W, Mourad MI, El-Aziz S, Elsabaa HM, Abou Elkhier MTJIJoAR. Evaluation of Caspase 3 as a Target for Apoptosis induced via Chemotherapy in Rats. 2015;3:1591-601.
60. Zhang JV, Jahr H, Luo C-W, Klein C, Van Kolen K, Ver Donck L, et al. Obestatin induction of early-response gene expression in gastrointestinal and adipose tissues and the mediatory role of G protein-coupled receptor, GPR39. 2008;22(6):1464-75.
61. Zhang JV, Ren P-G, Avsian-Kretchmer O, Luo C-W, Rauch R, Klein C, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. 2005;310(5750):996-9.
62. Zhang N, Yuan C, Li Z, Li J, Li X, Li C, et al. Meta-analysis of the relationship between obestatin and ghrelin levels and the ghrelin/obestatin ratio with respect to obesity. 2011;341(1):48-55.

Table I. Colonic injury grades and number of goblet cells among the study groups observed 14 days after induction of colitis.

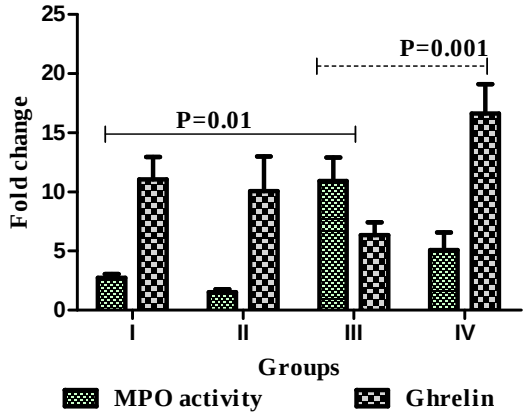
Groups	**I**	**II**	**III**	**IV**
Microscopic grade	0.89±0.2	0.92±0.1	13.6±1.8[a]	5.3±2.6[b]
Goblet cell	51.3±12.5	50.9±11.9	18.7±12.3[a]	42.8±15.2[b]

Mean ± Standard deviation (SD). n = 10 in each group of animals. [a]P< 0.01 (group III vs group I), [b]P< 0.001 (group III vs group II).

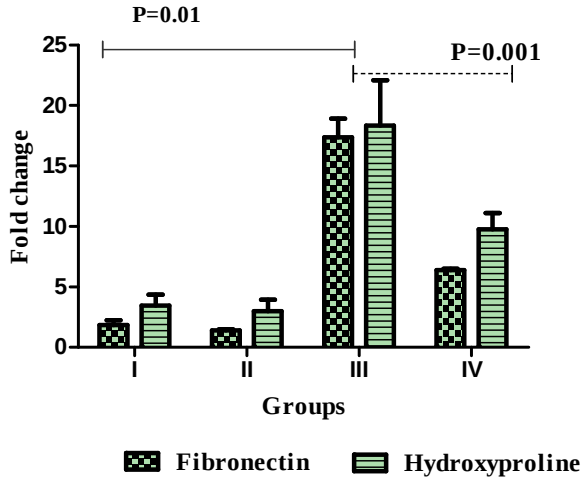
Table II. Comparison of tissue oxidant-antioxidant parameters among the study groups (n=10).

Groups	**I**	**II**	**III**	**IV**
MDA(nmol/g)	4.1±0.46	3.52±0.38[a]	7.5±2.8[b]	3.6±1.3 [c]
TOS (micromole/g)	1.86±1.2	1.76±1.5[a]	4.8±12.3[b]	2.8±1.4 [c]
SOD (U/mg protein)	2.4±0.98	2.8±0.91[a]	0.51±04[b]	3.6±1.9[c]
CAT (k/g protein)	1.8±0.65	1.9±0.84[a]	0.36±0.45[b]	1.6±1.1[c]
TAC (m mole trolox Eq/1)	2.1±0.48	2.8±0.25[a]	0.45±0.25[b]	2.3±1.4 [c]

Mean ± Standard deviation (SD). n = 10 in each group of animals. [a]P< 0.01 (group II vs group I), [b]P< 0.001 (group III vs group II /group I), [c]P< 0.003 (group IV vs group III). MDA: malondialdehyde; TOS: total oxidant status; SOD: superoxide dismutase; CAT: catalase; TAC: total anti-oxidant capacity.



Graph 1. Levels of MPO and ghrelin activity in colonic mucosa of rats in control and experimental groups. In rats with colitis (group III), significant increase in the activity of tissue MPO with a reduction in the levels of tissue ghrelin were observed ($p < 0.01$). Group IV presents a significant reduction in the activity of MPO with an elevation in the levels of tissue ghrelin ($p < 0.001$) following the treatment with obestatin. The results expressed as Mean ± standard deviation (SD). n = 10 in each group of animals. $p < 0.01$when compared with group I; $p < 0.001$ when compared with group I or II& III respectively.



Graph 2. The expression of fibrotic markers (Fibronectin and Hydroxy proline) in control and experimental groups. Rats treated of group III shows significant increase in the expression levels of fibronectin and hydroxyproline compared to control group ($P< 0.01$). However, group IV shows a significant reduction in the expression of both fibronectin and hydroxyproline compared to group III ($P < 0.001$). The results expressed as Mean ± standard deviation (SD). n = 10 in each group of animals. $P< 0.01$when compared with group I or II; $P < 0.001$ when compared with group III.

Figure 1. Tissue sections of rats in group I and II stained with hematoxylin and eosin exhibited normal colonic structure with intact colonic mucosa and numerous goblet cells on the surface epithelium and crypts (Figs. IA & B). Fig. C (group III) shows marked pathological changes in all colonic laminae which appears barely differentiated. The mucosa shows focal ulceration with complete absence of the goblet cells. The muscularis mucosae are not evident and the submucosa is infiltrated by extensive inflammatory cell infiltration. Group IV shows areas of limited ulceration, inflammatory cell infiltration and loss of crypts alternating with areas of intact mucosa with goblet cells and crypts revival (Fig. 1D).

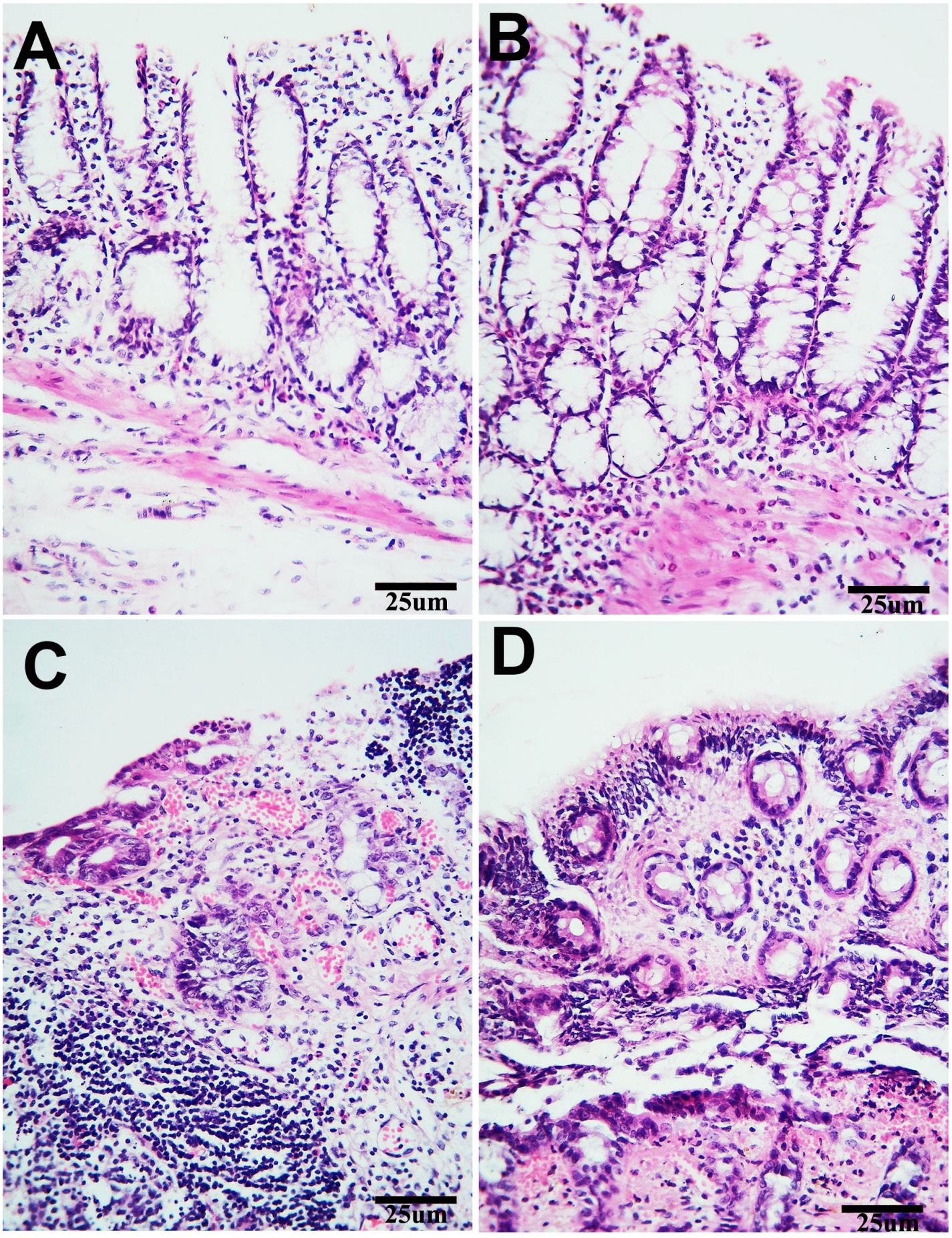
Figure 2. The mucous secretions of the goblet cells in groups I & II stained Alcian blue demonstrated numerous deeply blue stained goblet cells lining the crypts and intact film of mucus on the surface epithelium (Fig. 2A & B). Fig. 2C (group III) shows nearly absent goblet cells and mucous in addition to extreme reduction in the intensity of the stain. Group IV (Fig.2D) revealed few goblet cells and restored intact film of mucus on the surface epithelium.

Figure 3. Masson trichrome stain of groups I & II. Minimal bluish stained collagen fibers can be detected in the lamina propria, muscularis mucosae, and the submucosa (Fig.3 A & B). Fig.3C (group III) shows increased expression of bluish stained collagen fibers in all colonic laminae. Fig.3D (group IV) show extensive collagen fibers expression in the submucosa and limited fibers in the mucosa.

Figure 4. The immunohistochemical examination of PCNA of the mucosa of the colon of group I demonstrates weak brown cytoplasmic positive immunoreaction in the epithelium and few cells in the lamina propria (Fig. 4A). The picture is more or less the same in group II (Fig. 4B). Group III shows marked increased immunoreactivity in both epithelial cells and cells of lamina propria (Fig. 4C). Group IV reveals decrease in positive immunoreaction (Figure 4D).

Figure 5. NFKB immune staining of the group I show scanty brownish cytoplasmic positive immunoreaction in both the surface epithelium and the cells of lamina propria (Fig.5 A). Group II showed a more or less similar presentation in the mucosal crypts (Fig. 5B). Group III displays widespread marked immunoreactivity in all layers of colonic mucosa (Fig. 5C), while colonic mucosa of group IV presents a limited appearance of the immunoreactivity to the mucosa and nearly absent reaction in the lamina propria (Figure 5D).

Figure 6. Caspase 3 expression in the colon mucosa showing nearly absent reaction in both groups I & II (Fig. 6 A & B). Group III shows strong and diffuse reaction in all layers of the colonic mucosa (Fig. 6 C). Group IV on the other hand shows very weak reactivity (Fig. 6D).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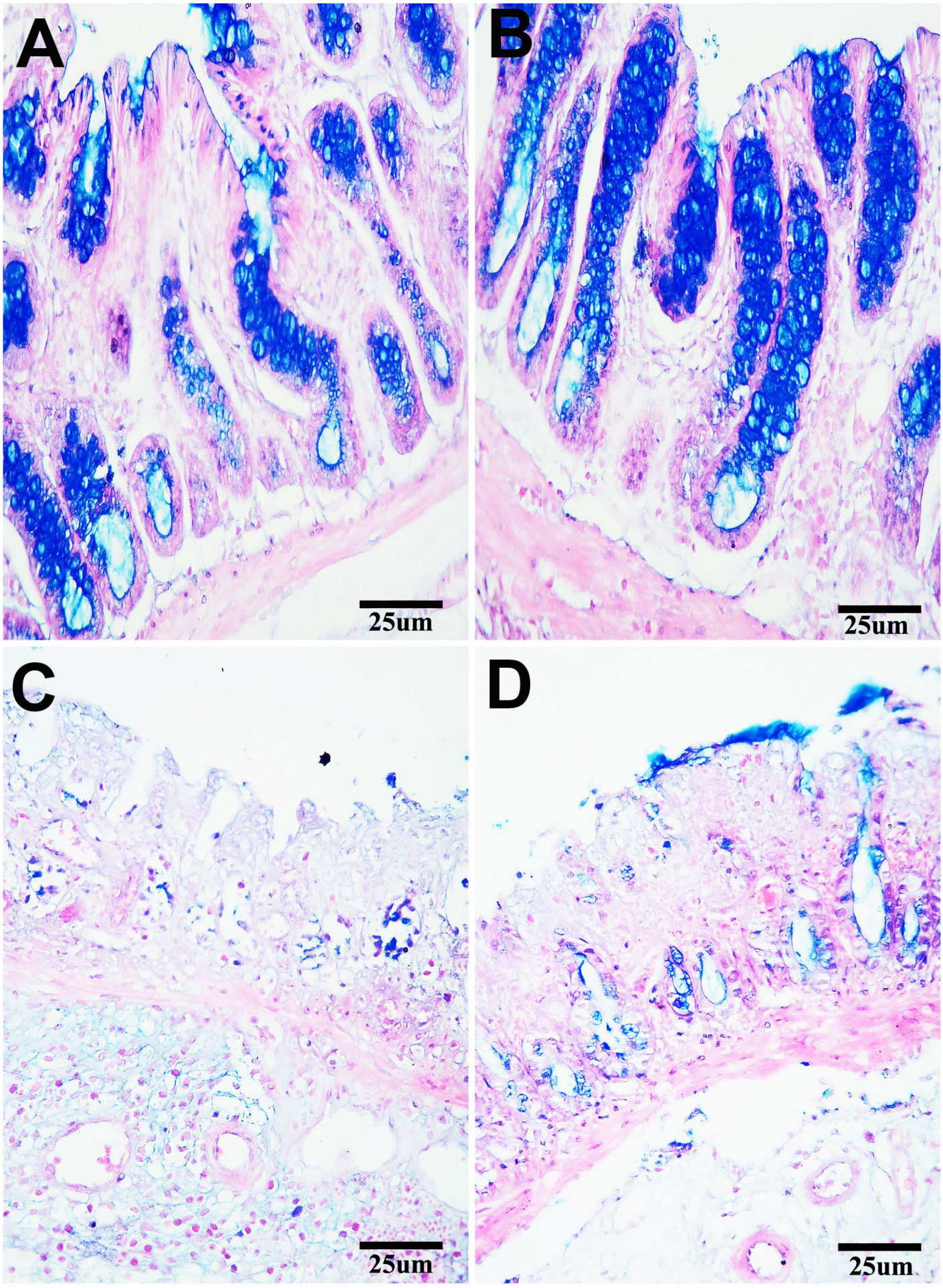
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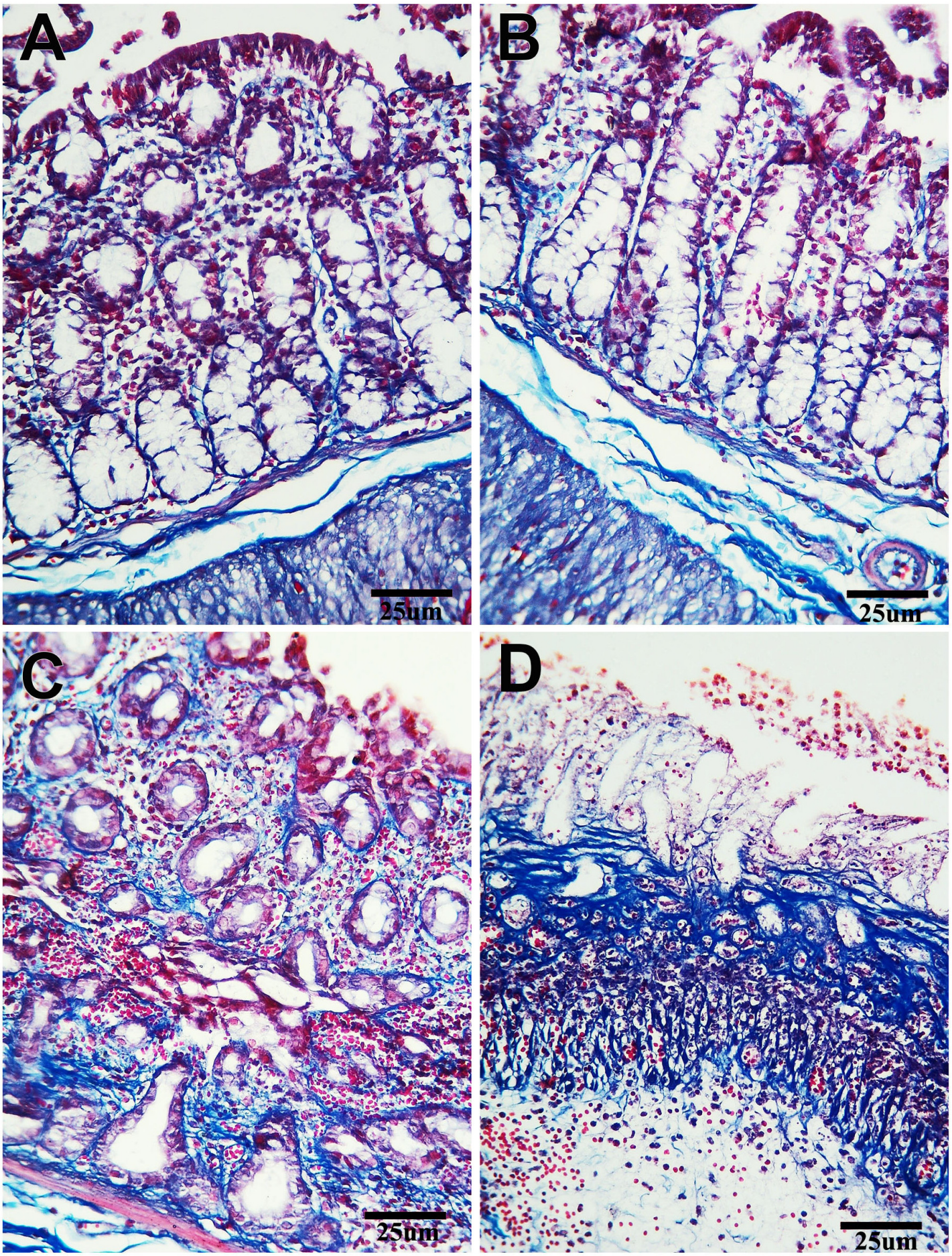
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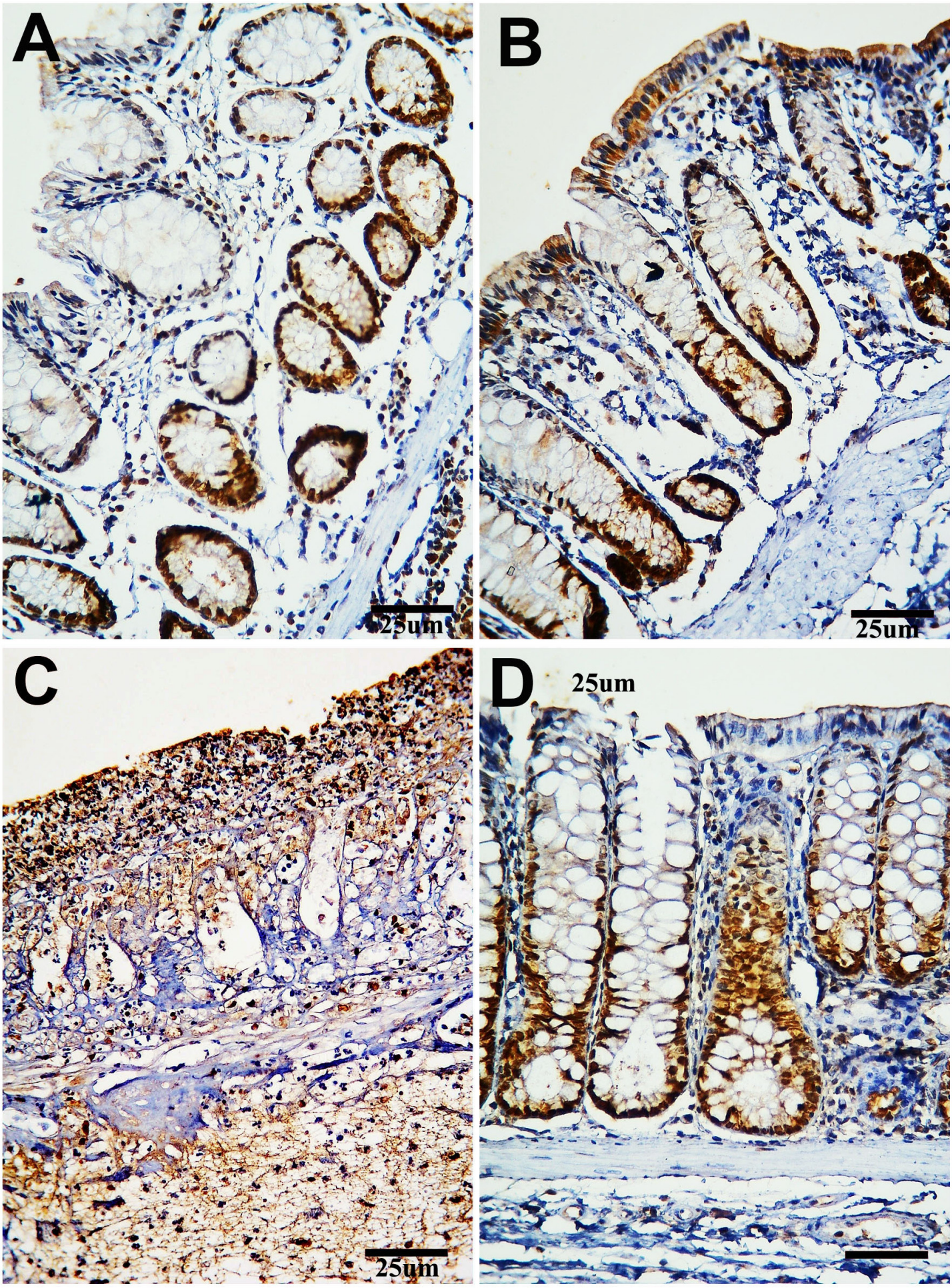
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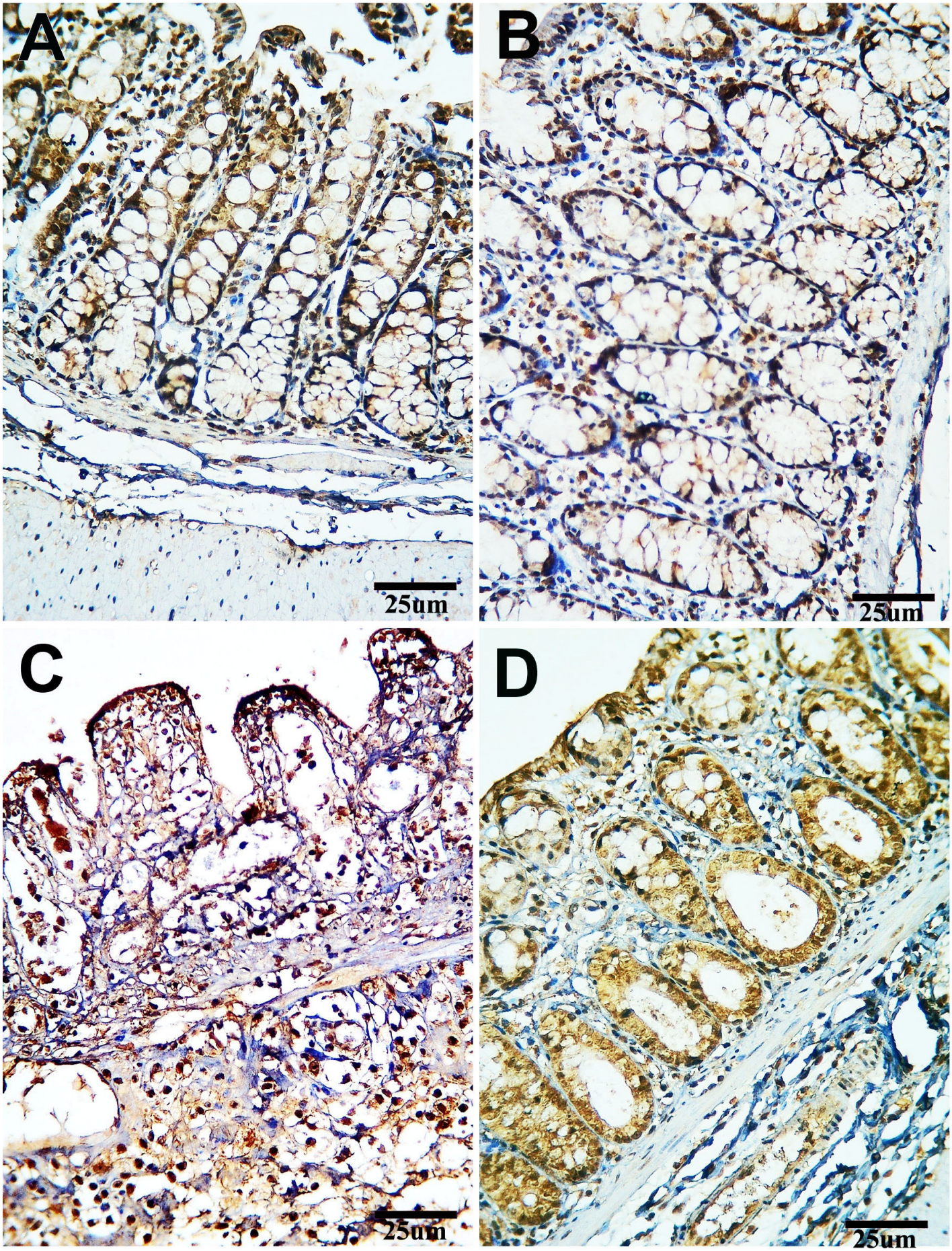
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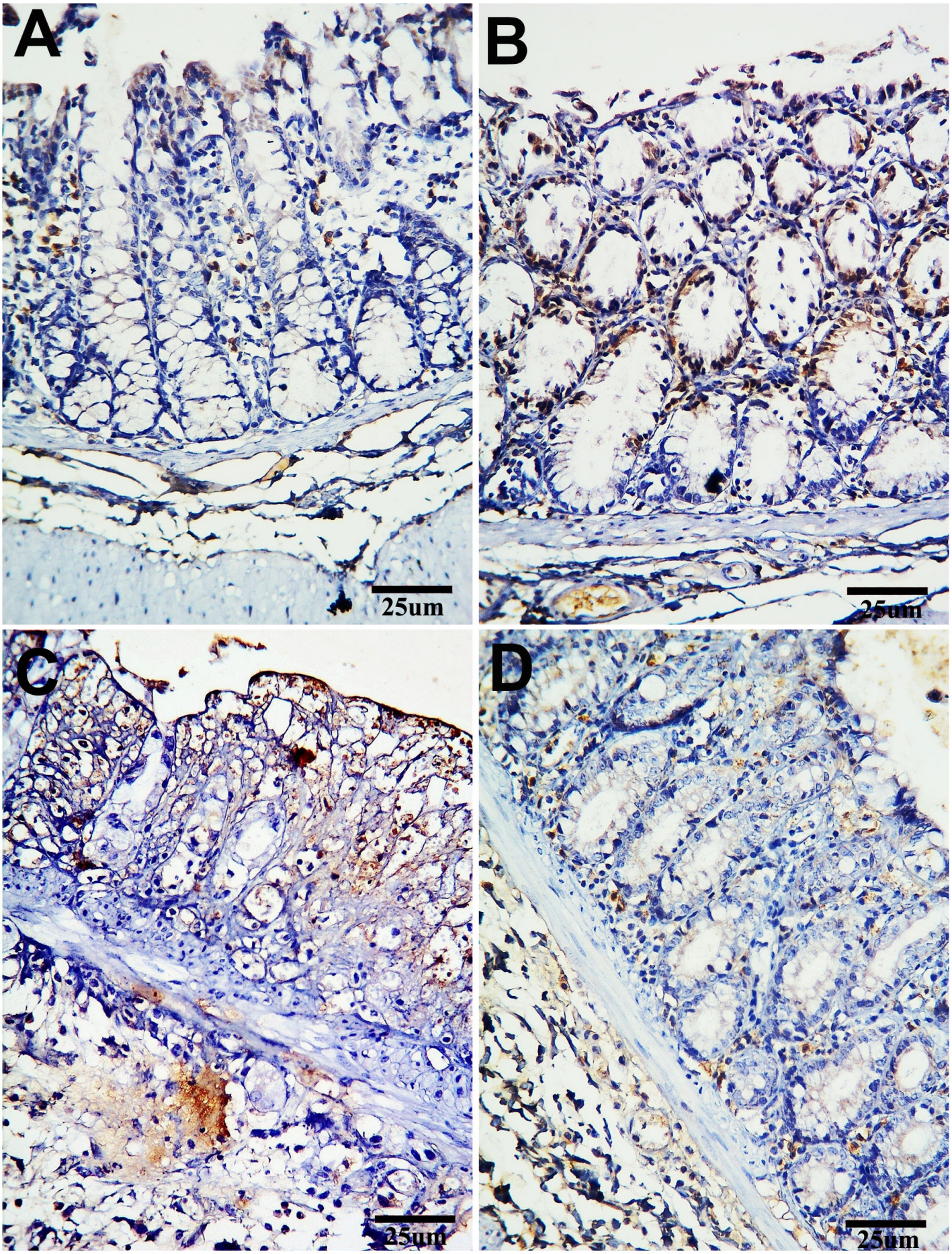
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